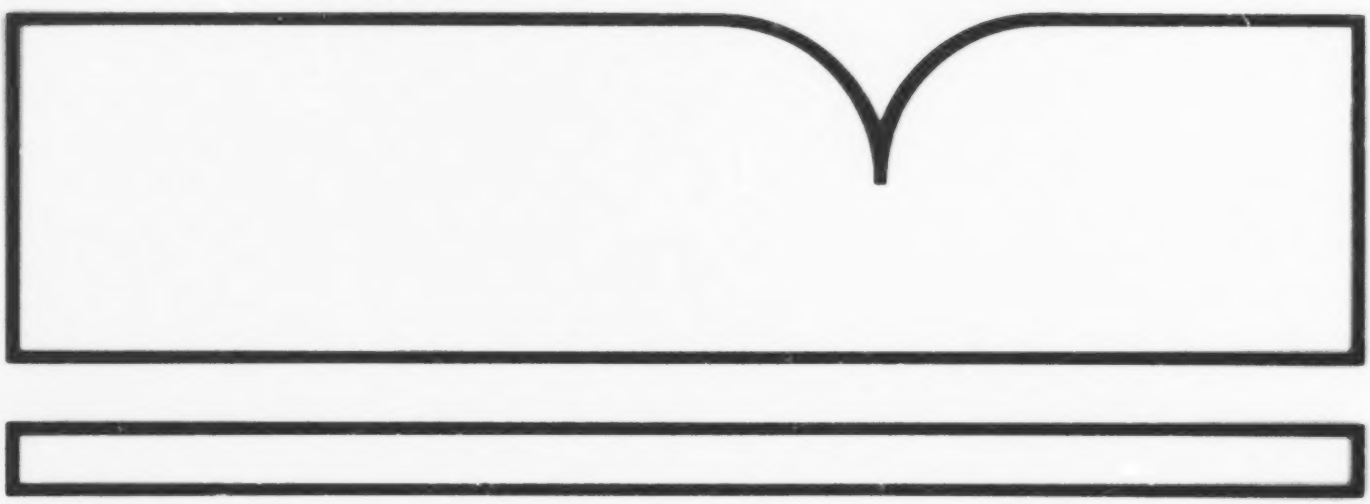
PB86-116571

Assessing the Biodegradation of Sediment Associated Chemicals

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

Oct 85



U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-116571

EPA/600/D-85/233
October 1985

Assessing the Biodegradation of Sediment Associated Chemicals

by

P.H. Pritchard
U.S. Environmental Protection Agency
Gulf Breeze, FL 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/233		PB86 116571/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
ASSESSING THE BIODEGRADATION OF SEDIMENT-ASSOCIATED CHEMICALS	October 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
P.H. Pritchard	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Same as #12	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory - Gulf Breeze, FL Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561	14. SPONSORING AGENCY CODE EPA/600/4
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

Investigations of the fate of xenobiotic chemicals in laboratory systems that accommodate the microbial ecology of sediments are described. These systems permit examination of biochemical activities in the sediment bed with particular emphasis at the sediment-water interface. Sediment may contain thousands of microcommunities, each containing the same genotypic array of metabolic potential. Each community, however, will demonstrate, depending on the surrounding conditions, a certain phenotypic response that reflects a small portion of its total metabolic potential.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS OPEN ENDED TERMS	c. COSATI Field Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	54
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

DISCLAIMER

The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to Agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

INTRODUCTION

Monitoring studies and laboratory investigations have demonstrated that many xenobiotic chemicals become readily associated with sediments. Sediments are known to harbor communities of microorganisms with very diverse metabolic capabilities. We expect, therefore, that some of these chemicals will be biodegraded. Surprisingly little qualitative and quantitative information exists on the biodegradation of pollutants in sediments. As Bull (3) stated, "Most biodegradation studies have not, or only incidently have considered interface effects; the importance of surfaces, films and aggregations remains poorly understood." This lack of information is a major stumbling block in hazard assessment procedures because the exposure of sensitive plant and animal species to a chemical cannot be fully estimated in sediments. In the past, this problem was circumvented by assuming that highly hydrophobic chemicals are generally resistant to biodegradation. Thus, exposure could be described by sorption rates and partitioning. We now realize that these assumptions are not entirely correct. Many hydrophobic chemicals are, in fact, biodegradable. I contend, based on new information concerning the vigor and diversity of biodegradation processes in sediments, that the association of a chemical with sediment may actually enhance degradability and decrease exposure to a potentially threatened organism. The purpose of this paper is to summarize applicable literature and research which supports this contention.

Studying the biodegradation of xenobiotic chemicals in sediments is a fascinating and complex undertaking. For example, we do not know if chemicals are degraded in the sorbed state or in interstitial water or a combination thereof. If a chemical is degraded in the sorbed state, how does it physically get to the catalytic site? Or does it sorb only to

the catalytic site? We are just beginning to investigate the sediment-associated bacteria as a potentially unique group of surface-loving organisms. If they exist, are their biodegradation capabilities different from those of microbial communities unassociated with sediment? As I will demonstrate below, the biodegradation rate of xenobiotic chemicals in sediments is frequently quite high relative to rates in water. In certain instances this is simply a microbial biomass effect while in others it may be a surface-related enhancement of degradation. Kinetic descriptions for biodegradation processes in sediments have been addressed infrequently.

Finally, the environmental factors affecting the biodegradation of xenobiotic chemicals in sediments have been virtually untested. The availability of the final electron acceptors (O_2, NO_3 SO_4, and CO_2) and their variability in time and in space (e.g., as assessed by Eh measurements) undoubtedly will affect biodegradation rates. Processes such as diffusion, bioturbation, and nutrient cycling will in turn control Eh gradients. To adequately study these factors, laboratory systems need to be developed that will maintain the sediment in as natural a state as possible and at the same time allow quantitation of biodegradation processes. These systems should permit examination of biochemical activities in the sediment bed with particular emphasis at the sediment-water interface. Sediment may contain thousands of microcommunities, each containing the same genotypic array of metabolic potential. Each community, however, will demonstrate, depending on the surrounding conditions, a certain phenotypic response that will be a reflection of only a small portion of their total metabolic potential. A particular disturbance to these communities, which may occur in certain experimental studies, could change the natural expression of metabolic activity and ultimately give misleading assessments

of biodegradability. Therefore, I believe the assessment of biodegradation processes in sediments requires some familiarity with the microbial ecology of sediments. I will describe experiments dealing with the fate of xenobiotic chemicals in laboratory systems designed to accommodate some of these ecological aspects.

Biodegradation in Sediments

The fate of pesticides, herbicides and other chemicals in ponds, flooded soils, and various laboratory sediment (soil)/water systems, has been studied numerous times but little attempt has been made to distinguish losses of parent compounds due to biodegradation from losses due to dilution, sorption, hydrolysis, diffusion and photolysis. Rapid losses of parent compound during these types of studies is often attributed to biodegradation in sediment only by the process of elimination; i.e., no other fate processes are considered likely. For example, Sikka and Rice (37) followed the fate of endothall in a farm pond and in laboratory microcosms. They attributed the observed decline of endothall residues in sediment to biodegradation processes therein, since it was unlikely, based on other information, that hydrolysis, photolysis or volatization would affect the fate of the endothall. Hexachlorobenzene, pentachloronitrobenzene and 4-chloroaniline, when applied to experimental ponds were also found to slowly accumulate in sediments and then slowly decline (36). Although, the authors did not specifically discuss degradative processes in the sediments, undoubtedly their results suggest this possibility. Caplan et al. (4) inferred, that the fate of fenvalerate (a pyrethroid insecticide with high affinity for surfaces) in a tidal salt marsh microcosm was due to biodegradation in sediment. They detected breakdown products in the sediments that were not typical of photolysis or abiotic degradation. However, Permethrin, another pyrethroid insecticide, was shown to accumulate and persist (470 days post treatment) in the hydrosoil of artificial outdoor ponds dosed with the insecticide (34). The detection of degradation products in the water column suggested that the permethrin was susceptible to degradation of some type. Persistence of the chemical in sediments cannot be

attributed necessarily to sorption to sediment surfaces since many other factors such as lack of degradation under anaerobic conditions could produce the same result. Chapman et al. (5) demonstrated that many types of the pyrethroids degrade in soil, but specifically fenvalerate degraded slower in organic soils than it did in mineral soils, suggesting that the sorption of this insecticide to organic matter possibly increased its persistence.

Other types of experiments have also indicated indirectly that microbial communities in sediments can be active in the biodegradation of xenobiotic chemicals. Graetz et al. (14) demonstrated that radiolabeled parathion rapidly degraded in active but not sterilized sediment-water slurries from a lake. The authors made no attempt to access biodegradation in water alone and thus the involvement of sediment-associated microorganisms in the biodegradation process can only be inferred. Amino parathion, a reductive transformation product of parathion, was the principal degradation product observed. Formation of this byproduct seems more typical of sediments where anaerobic conditions commonly found, rather than the highly oxygenated water. Pentachlorophenol (PCP) degradation, as evidenced by rapid mineralization to CO_2, in sediment-water slurries taken from the Mississippi River/Gulf outlet, was observed by DeLaune et al. (8). Mineralization of radiolabeled parent compound to CO_2 was faster in sediments previously exposed to PCP as a result of a spill. Again, the extent of PCP degradation in water alone was not determined. Pignatello et al. (31) have more definitely characterized the potential for PCP degradation in sediments. The mineralization capability of sediment-water slurries taken from a man-made stream channel, continuously dosed with PCP, dramatically increased at about the same time that PCP losses increased in the stream channel.

In other similar studies, toxaphene-contaminated sediments slowly lost their toxicity, presumably because of microbial activities (24). Several investigations have also detected toxaphene transformation products in sediments both under aerobic and anaerobic conditions (7,40,49). Kong and Sayler (20) isolated pure cultures of bacteria from river sediments (although there is nothing unique about these isolations to claim that they are sediment inhabitants as opposed to water inhabitants) that degraded several types of monohalogenated biphyenyls. Bacterial cultures that metabolize the pesticide, dichlobenil, have been isolated from lake sediments (27). Thus, the microbial communities associated with sediments appear to have significant metabolic capabilities for a variety of chemical structures.

The importance of biodegradation processes in sediments is even more apparent if the quantitative aspects are examined. Several studies recently have revealed that biodegradation rates of xenobiotic chemicals in sediments greatly exceed those observed in water (without sediment). This is not surprising, judging from the large numbers of studies which have shown much more rapid turnover of simple natural organics (amino acids, glucose, organic acids) in sediments than in water (6,14,19,23). Lee and Ryan (23) were the first group to carefully examine the effects of sediments on the biodegradation kinetics of xenobiotic chemicals. The mineralization rates of radiolabeled xenobiotic chemicals were measured in estuarine water with and without sediments. A first order kinetic expression adequately fit their mineralization data and allowed them to calculate half-lives of p-chlorophenol, trichlorophenol, chlorobenzene and trichlorophenoxy acetic acid (Table 1). All of the chemicals degraded slowly in water. The presence of sediment (50 gm/l) decreased the half-

Table 1. Degradation of radiolabeled compounds in Skidaway river water and sediments[a]

Water

Compound	Water temperature (°C)	Rate constant, k ($days^{-1}$)	Half-life $t^{1}/^{2}$ (days)
p-Chlorophenol	21	.035	20
2,4,5-Trichlorophenol	21	.0010	690
Chlorobenzene	22	.0045	150
1,4,5-Trichlorophenoxy-acetic acid	22	.0005	1400

Water-sediment slurry

Compound	Water temperature (°C)	Rate constant, k ($days^{-1}$)	Half-life, $t^{1}/^{2}$ (days)
p-Chlorenophenol	22	.23	3
2,4,5-Trichlorophenol	21	.030	23
Chlorobenzene	21	.0092	75
1,4,5-Trichlorophenoxy-acetic acid	21	.012	35

[a]Radiolabeled compounds were added to 100 ml water samples to give a concentration of 25 µg/liter. At the end of various incubation periods the $^{14}CO_2$ produced was collected and counted as a measure of the degradation. The rate constant for each compound was calculated from data fitted to a first-order rate expression.

From Lee and Ryan (23)

lives by 7, 27, 2 and 41 times, respectively.

The results of these studies are highly significant for two reasons: 1) Several of the xenobiotic chemicals studied would be classified as persistent environmental pollutants if biodegradation information was based solely on the activities of natural microbial communities in water. Rapid degradation in the presence of sediments clearly changes this classification; 2) The chemicals tested by Lee and Ryan (23) cover a wide range of partition coefficients. This suggests that biodegradation processes in sediments are just as effective against water soluble chemicals (low affinity for surface adsorption) as they are against more insoluble chemicals (high affinity for surface adsorption). Thus, when the biodegradation of sediment-associated chemicals is discussed, the term "associated" should be considered as any mixing of the chemical with sediment. The term should not necessarily connotate a high degree of sorption to sediment surfaces.

Several other investigations have shown faster degradation rates of xenobiotic chemicals in water and when sediments are present. Baker et al. (1) reported sediment-enhanced degradation rates of chlorophenols in samples from a field stream. The mineralization rates of several types of detergents in natural water samples has been shown by Larson (21, 22) to be stimulated by the addition of sediments. Van Veld and Spain (44) examined the biodegradation rates of p-nitrophenol, p-cresol and methylparathion in shake flask systems containing sediment slurries from two estuarine sites and found half-lives to be consistently larger whenever sediments were excluded from the test systems. Decay curves for methyl parathion and p-nitrophenol that exemplify this sediment effect are shown in figure 1. Commencement of p-nitrophenol degradation (adaptation) was

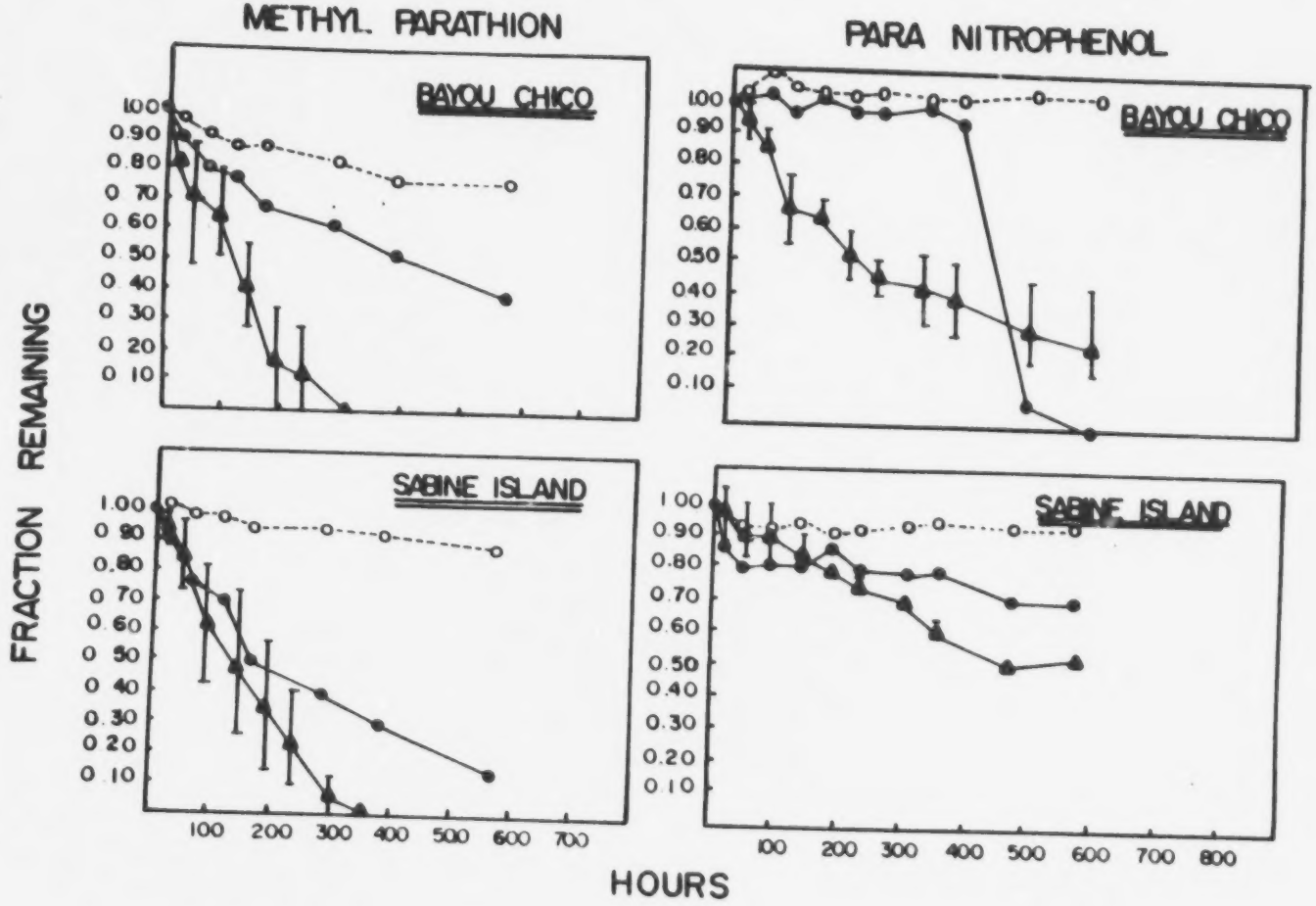


Figure 1. Comparison of decay curves for methyl parathion and p-nitrophenol between shake flask studies with (closed circles) and without (open circles) sediment slurries and eco-core studies (triangle) with intact sediment cores. From Van Veld and Spain (38).

only observed (over this incubation period) when sediments were present. Curiously, degradation of both p-nitrophenol and methyl parathion in eco-cores (33, 2), i.e., with intact sediment cores, was the fastest and commenced without lag. First order rate constants for the degradation of naphthalene, anthracene and benz(a)anthracene in sediments have been shown by Herbes (15) to be 5-20 fold faster than rates rates observed in water.

Walker et al. (45) showed that sediments specifically enhanced the biodegradation of dibutylphthalate. The biodegradation of a variety of pesticides in samples from various aquatic environments, both estuarine and freshwater was more rapid when sediments were included (9,18,38,45). Experiments by Ward and Matsumura (47) suggest that the only way the persistent chemical, dioxin, will degrade in aquatic systems are when active lake sediments are present. Therefore, the accumulating evidence supports the idea that once a chemical becomes associated with sediment by whatever mechanism, the chances of the chemical being biodegraded could be considerably enhanced.

Several isolated studies have suggested that the association of xenobiotic chemicals with sediment may, however, actually decrease chances for biodegradation. This could be the result of several factors. The chemical structure of the compound in question may preclude biodegradation, regardless of whether the chemical is in water or in sediment. For example, the pesticide, permethrin, accumulated in the sediments of a pond dosed with the pesticide (34). Losses of the pesticide from the water column were quite rapid and the detection of several degradation products in the water column suggested that photolysis was responsible for some of the product formation. Low light levels at the sediment surface probably

resulted in little photolytic degradation of the permethrin sorbed to sediments. The role of biotic transformation was not clearly delineated. Possibly, the chemical was resistant to biodegradation by microbial communities in water regardless of the presence of sediment. The accumulation of fluridone, an experimental herbicide, in pond sediments relative to its rapid degradation in pond water (28), may also reflect the absence of photolysis in sediments and not the absence of biodegradation potential. Paraquat and diquat, herbicides which strongly sorb to sediments, are known to be biodegraded by soil microorganisms (48). A study using a simulated lake impoundment (39), showed that diquat accumulated and persisted in sediments. Sorption to the sediment surfaces through ionic interaction (a characteristic of this pesticide class) may have prevented biodegradation. Indeed, Weber and Coble (48) have shown that microbial decomposition of diquat was proportionally decreased as more and more clay mineral was added to the test systems. An example of their data is shown in table 2. Enough clay in fact, could, be added to completely stop degradation. These results may be unique for the dipyridinium herbicides primarily because of sorption characteristics. Increasing sorptive surfaces by adding more sediment may not necessarily have the same effect as adding azooic clays since more microorganisms (and possibly biodegradative potential) would be added along with the sediments.

The fate of di-2-ethylhexylphthalate (DEHP) in microcosms designed to simulate a portion of Narragansett Bay (Rhode Island), has been examined by Perez et al. (30). They observed that DEHP readily biodegraded in the water column but accumulated to relatively high amounts in the sediments (Table 3). Mass balance for the distribution of the DEHP suggests that sorption to sediment surfaces prevented biodegradation. Even DEHP sorbed

Table 2a. Cumulative release of $^{14}C)_2$ resulting from application of radiolabeled diquat to nutrient solutions containing montmorillonite (M) and kaolinite (K)clays

Diquat added (µMoles)	Clay added (Mg.)	EVOLVED $^{14}CO_2$ CPM[a]				
		1 week	2 weeks	4 weeks	6 weeks	8 weeks
3.7	0	37,000	80,800	159,000	217,000	254,000
3.7	5 M	16,300	27,300	44,300	60,200	72,800
3.7	10 M	0	0	0	0	0
3.7	80 K	38,800	58,700	106,000	150,000	187,000
3.7	170 K	24,400	46,000	94,500	152,000	204,000

[a] Corrected for $^{14}CO_2$ derived from sterile control (<200 CPM)

Table 2b. Tagged diquat remaining in nutrient solutions and in montmorillonite (M) and kaolinite (K) clay pellets at termination of experiment 3

Diquat Added (µCi)	Clay Added (Mg)	C^{14} -Diquat Present[a] (µCi)	
		In Solution	In Pellet[b]
10	0	8.4	0.4
10	5 M	5.1	4.5
10	10 M	0	9.7
10	80 K	8.1	1.0
10	170 K	5.4	3.6

[a] Corrected for background
[b] Pellet contained microbial cells and clay.

From Weber and Coble (42)

Table 3. Mean concentrations of DEHP in the water column ($\mu gm(kgm\ dry)^{-1}$) during the winter (W) and summer (S) seasons of marine microcosms at the end of 30-day incubation period.

		INITIAL WATER COLUMN (DEHP)		
Source	Season	1	10	100
Water Column	W	0.56	4.44	45.76
	S	0.15	0.93	9.83
Sediment	W	45.3	225.8	4,737.9
	S	53.4	225.6	3,377.5

FROM PEREZ ET AL. (30)

to the vessel wall was apparently resistent to the degradative activities of bacteria in the water. Johnson and Lulves (17), on the other hand, have shown, using flask studies with sediment-water slurry samples from a local freshwater pond, that DEHP is readily degraded. Since they did not measure the degradation rate of DEHP in pond water without sediment, it was possible that most of their observed biodegradation resulted from microbial communities in the water rather than those on the sediment particles. The results of Perez are, therefore, unique and merit further investigation.

Subba-Rao and Alexander (43) reported a similar effect on the mineralization of benzylamine; increases in clay concentration generally decreased the percentage of substrate mineralized. However, they showed a stimulation in mineralization at high benzylamine concentration (20 mg/l) when montmorillonite clays were present in the test systems. They suggest that mineralization rates were limited by the desorption rates of the benzylamine from the clay surface. Rates of methanearsonic acid demethylation to arsenic were shown by Holm et al. (16) to be inversely proportional to the concentration of the adsorbing species, such as clay minerals, and its adsorption capacity. Therefore sorption to particulate surfaces would seem to reduce (lowered initial concentration) rather than stimulate degradation rates. These types of results led Steen et al. (42) to develop a kinetic expression for the effect of sediments on biodegradation rates. Second-order biodegradation rate kinetics, rapid sorption relative to degradation, and biodegradation of only dissolved chemical were assumed. The expression was:

$$\frac{dS}{dt} = \frac{k[S]\,[B]}{1 + K_p[p]} \qquad (1)$$

where [S] = concentration of test chemical, [B] = total bacterial

concentration in water and sediment (measured as ATP), k = second-order rate constant, Kp = equilibrium partition coefficient of test chemical with respect to sediment and ρ = the sediment to water mass ratio. An example of their data for the biodegradation of chloropropham and di-n-butyl phthalate are shown in Table 4. Close agreement between second-order rate constants in the presence and absence of sediments led to them to conclude that sorption of the test chemical to suspended sediments render the compound unavailable for degradation (no degradation in the sorbed state). Degradation rate of parent compounds also was observed to be inversely related to sediment concentration (figure 2). ATP, which is a general measure of total microbial biomass, is used by Steen et al. (42) as a surrogate measure of the degrader populations (they assume degraders are always a constant fraction of the total population). Since ATP was presumably found in association with sediment, by definition, degraders should also be present on the sediment. If it is assumed, as Steen et al. suggest, that sorbed chemical is not degraded, the degraders on sediment apparently only metabolize dissolved substrate. This is reasonable considering that the biodegradation of many radiolabeled chemicals results in the accumualtion of radioactivity on sediments which cannot be attributable to sorption of parent compound (i.e., radioactivity can not be extracted with conventional solvents). This bound radioactivity is usually quantified by combustion to radiolabeled CO_2. Bound radioactivity does not occur in test systems sterilized with formalin. The major degradation products of certain pesticides (18, 33), substituted phenols (44), and polycyclic aromatic hydrocarbons (15) in sediment-water test systems, have been shown to be radioactive bound residues. Most investigators believe that the bound radioactivity is actually radiolabeled cellular components of

Table 4. Comparison of observed second-order microbial rate constants (k_{obs}) vs. second-order constants (k_2) affected by sediment partitioning.

Compound	Fraction Sorbed (Hickory Hill Sediment)	K_{obs} (liter $org^{-1} hr^{-1}$)	k_2 (liter $org^{-1} hr^{-1}$)
Chlorpropham (CIPC)	0	$1.42 \pm 0.6 \times 10^{-13}$	----
	0.15	$4.15 \pm 0.7 \times 10^{-13}$	$3.45 \pm 0.2 \times 10^{-13}$
	0.90	$7.4 \pm 1.2 \times 10^{-15}$	$2.29 \pm 0.6 \times 10^{-13}$
Di-n-Butyl Phthalate	0	$3.1 \pm 0.8 \times 10^{-11}$	----
	0.15	$2.7 \pm 1.5 \times 10^{-11}$	$3.47 \pm 1.7 \times 10^{-11}$
	0.90	$6.1 \pm 0.5 \times 10^{-13}$	$2.60 \pm 0.21 \times 10^{-11}$

From Steen et al. (42)

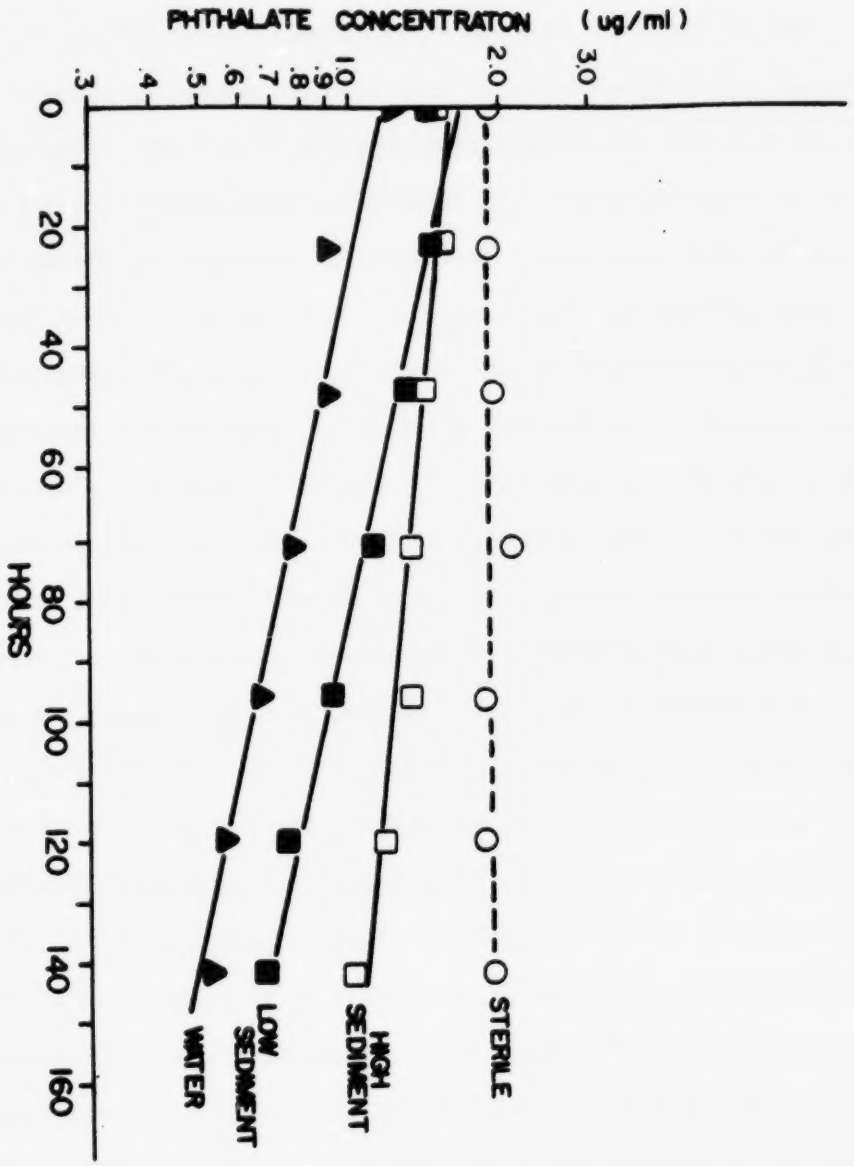


Figure 2. Pseudo-order plot microbial degradation of di-n-butyl phthalate in natural water and sediment-water systems. From Steen et al. (42)

bacteria on the sediment surface resulting from the biodegradation and carbon assimilation of the test chemical.

Studies in our laboratory by Dr. Paul Lefcourt on the biodegradation of di-n-butylphthalate indicate that in some environmental samples containing sediments, the kinetics may be more complex and not as easily explained by the relationship described by Steen et al. The biodegradation of di-n-butyl phthalate was examined in shake flask tests containing estuarine water and sediment (100 µg/ℓ) and varying amounts of sterile (oven dried) sediment. The decay curves for a typical experiment with 100 mg/ℓ nonsterile sediment and 100 mg/ℓ non-sterile sediment with 1900 mg/ℓ sterile sediment are shown in figure 4. Very little biodegradation of the phthalate was observed in flasks with just estuarine water. Table 5 shows that first order rate constants at a single concentration of nonsterile sediment and five different concentrations of sterile sediment were approximately equal, suggesting no pronounced effects due to partitioning. Our results are similar to those of Larson and Vashon (22), who showed that there was no relationship between the biodegradation rate of dialkyl quaternary ammonium compounds in systems containing sediments and the chemical's affinity for sediment. Rapid colonization of the sterile sediments might explain our results; if biomass increased in proportion to the added sediment, it may have increased the degradation rate to the point of exactly counteracting the decrease in rate due to increased sorbed concentrations. Unfortunately, changes in biomass were not measured in our experiments and further work is needed to verify this explanation.

An alternative method for assessing the effect of partitioning on biodegradation, assuming that sorbed chemical is not degraded and that the concentration of catalytic units does not change over the course of

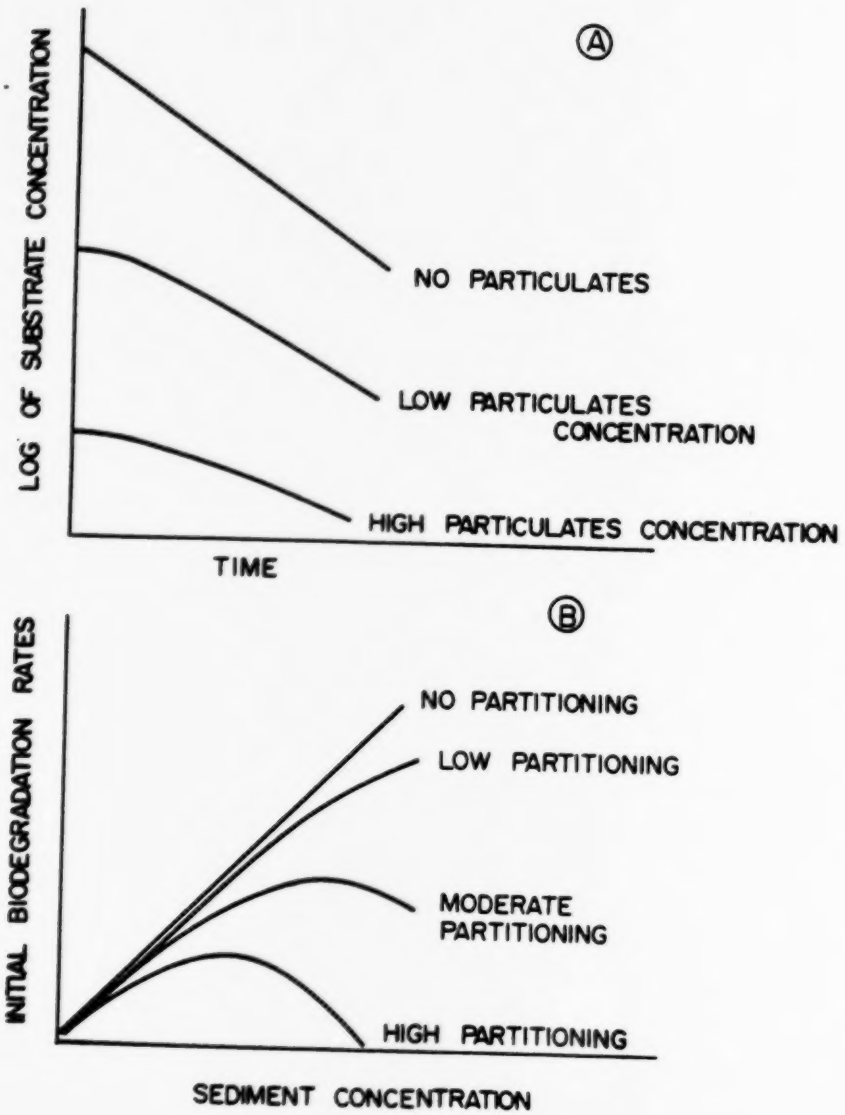


Figure 3. Decay curves of dissolved (dotted line) and total (solid lines) of di-n-butyl phthalate in shake flasks tests with estuarine water and sediment. Circular symbols refer to flask with 100 mg/ℓ nonsterile sediment and square symbols refer to flasks with 100 mg/ℓ nonsterile sediment and 1900 mg/ℓ sterile sediment.

Table 5. Effect of increasing sterile sediment concentrations on first order rate constants for phthalate biodegradation in estuarine water and sediment.

Total Sediment Concentration (mg/ℓ)[a]	Fraction[b] Sorbed	Dissolved Phthalate		Total Phthalate	
		k^c	r^{2d}	k^c	r^{2d}
100	0.05	-0.48	0.97	-0.56	0.92
300	0.25	-0.59	0.91	-0.42	0.96
500	0.37	-0.60	0.88	-0.60	0.92
1000	0.49	-0.65	0.86	-0.61	0.85
2000	0.57	-0.50	0.83	-0.50	0.77
4000	0.76	-0.19	0.61	-1.11	0.87

[a] Nonsterile sediment concentration in all tests was 100 mg/ℓ. Remainder was made up of sterile sediment (oven dried).

[b] Fraction sorbed equals the total phthalate concentration minus the dissolved phthalate concentration divided by total phthalate concentration.

[c] K are first order rate constants based on slopes from linear regression lines of semi-log plots of dissolved and total phthalate disappearance with time.

[d] Linear regression coefficients.

the experiment, would be to examine "initial" rates of degradation in the presence of sediment since partitioning will affect starting concentrations. For the purpose of discussion, dissolved and sorbed chemical are assumed to be at equilibrium at the beginning of an experiment. Initial rates, in this case, are operationally defined as a significant decrease in the concentration of parent compound over a period of a few hours. Degradation rates will be directly related to starting dissolved concentration of the test chemical if the rates are measured over a time period in which very little of the total substrate has been converted to product. Theoretically, in experiments dealing with partitioning of the chemical to sediment, initial rates will decrease as the fraction sorbed increases.

Many chemicals, however, will desorb from sediment at a rate considerably faster than biodegradation rates and there will be a span of time when degradation kinetics are essentially in steady state because test chemical concentrations will be maintained by desorption (over short periods) at approximately initial levels. Operationally this will appear as a lag in the disappearance of parent compound relative to tests without sediment. The use of high sediment concentration and/or chemicals with high partitioning will mean that most of the chemical, at equilibrium, will be in the sorbed state and starting concentrations in the water will be initially maintained without significantly affecting total substrate concentration. This is illustrated, hypothetically, in figure 3a. Absence of this lag in the disappearance of parent compound under these conditions would mean that either desorption is not much faster then the biodegradation rate or that biodegradation of the sorbed chemical was occurring.

In addition, the relationship between initial degradation rates and

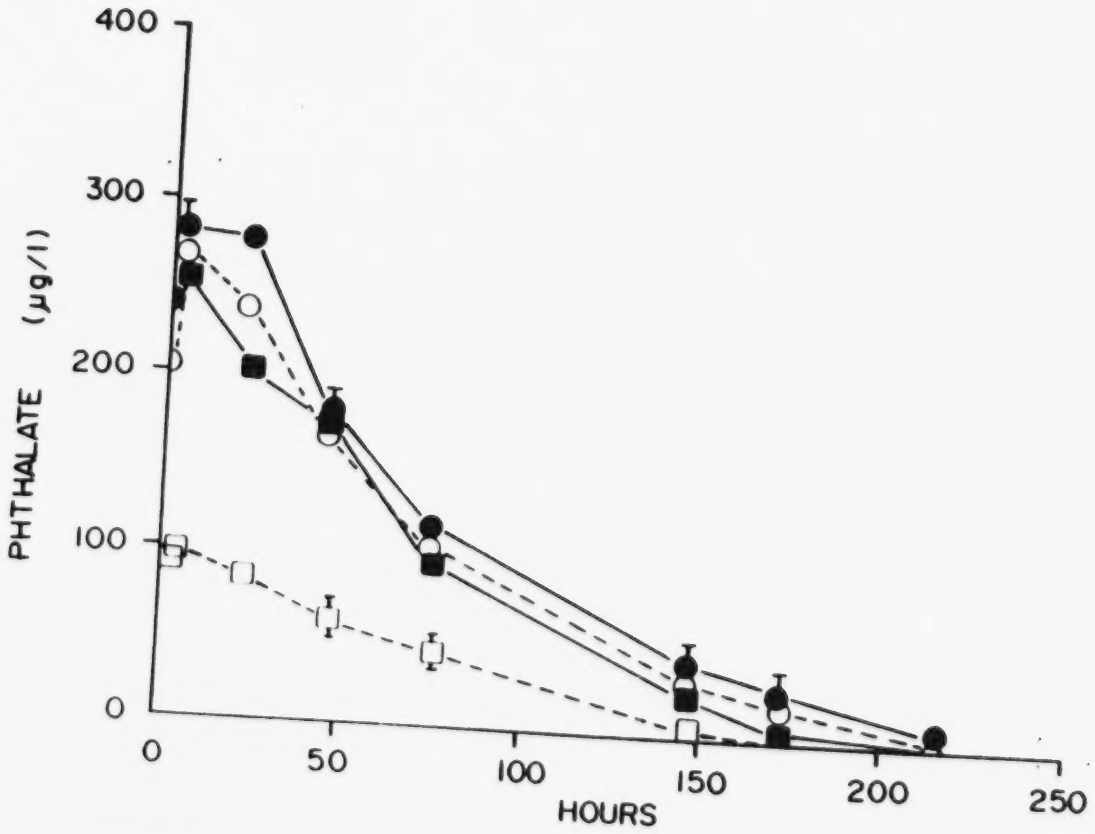


Figure 4. Hypothetical curves representing the effect of sorption and sediment concentration on biodegradation rates.

A. Effect of particulates concentration assuming biodegradation of dissolved chemical only and no microbial biomass on particulate material. Solid lines; desorption rates very rapid relative to biodegradation rates. Dotted lines; desorption rates approaching biodegradation rates. Slopes of respective lines are equal.

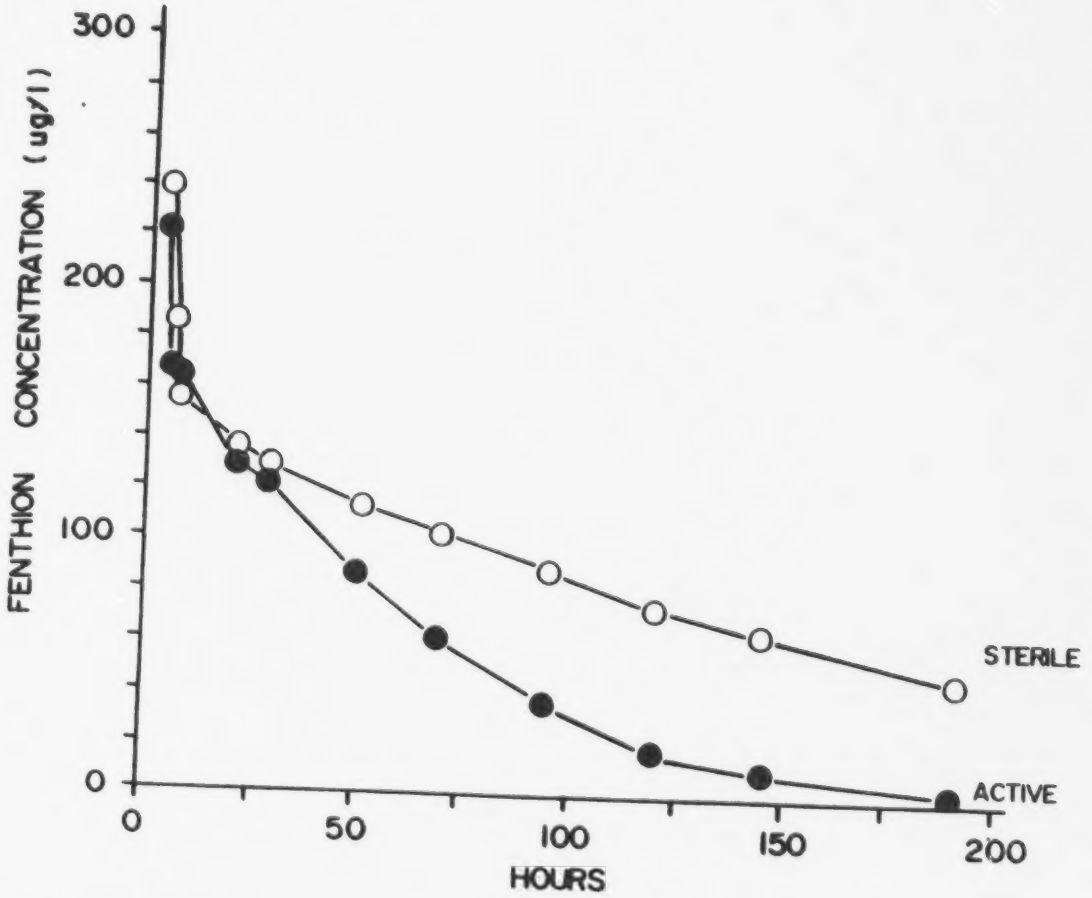


Figure 4. Hypothetical curves representing the effect of sorption and sediment concentration on biodegradation rates.

B. Effect of compounds with different partition coefficients assuming biodegradation of dissolved chemical only and microbial biomass on sediment is capable of degrading dissolved chemical.

sediment concentration assuming active degrader populations on the sediment, will not be linear, unless k_p, the partition coefficient, is zero (i.e., degradation rates are directly related to the biomass associated with the sediment). This is shown in the hypothetical plot in figure 3b (sorption equilibrium is assumed as a prequisite). Chemicals with small partition coefficients will give plots that appear linear at low sediment concentrations (i.e., most of the chemical is in the dissolved state) but that deviate from linearity at higher sediment concentrations. As partition coefficient increases from one chemical to the next, the biomass effect will be dwarfed because almost all of the chemical will be initially sorbed. If biodegradation of the sorbed chemical occurs, the effect of partitioning will be less pronounced and may even result in a completely linear relationship between initial degradation rates and sediment concentration. The control situation in this case would have to be a theoretical calculation based on equilbrium partitioning to sterile sediments.

Factors Affecting Biodegradation in Sediments

The more rapid biodegradation occurring in sediment systems versus water systems can be attributed to either a microbial biomass effect or a surface-catalysis effect. A biomass effect means that the bacteria capable of degrading the xenobiotic chemical (hereafter referred to as degraders) are more numerous on the sediment surface than they are in the surrounding water. Adding sediments to a water sample is essentially the same as inoculating the water with a culture of the degraders. Sediments provide a surface for the concentration of bacterial cells and the larger the number of cells, the more likely a significant number of degraders will be present to effect the fate of a xenobiotic chemical. Although conditions will vary considerably from one environment to another, in general, the total biomass of bacteria in the top 0.2 to 0.3 cm of a sediment bed, in shallow estuarine areas, may be 100-10,000 times higher than the total bacteria in the water column above the sediment (28). This would mean a similar magnitude difference in the degrader populations (assuming they are a constant proportion of the total). Experimental verification of this biomass effect is difficult because of problems in quantitating numbers of degraders on sediment. However, initial studies in our laboratory with p-chlorophenol (unpublished results), a chemical which does not readily degrade in seawater samples from Santa Rosa Sound unless sediments are present, have shown that p-chlorophenol degraders are more frequently isolated from microbial communities associated with sediment than they are from communities in the water column. Thus, the enhanced degradation of chlorophenol in sediment may be a biomass effect. Sediment-associated microorganisms are operationally defined, in this case, as those remaining on sediments after vigorous washing with sterile

seawater but released from sediments by repeated treatment in a homogenizer. Despite the lack of degradation of p-chlorophenol in natural seawater, degraders do exist in seawater as evidenced by the occassional isolation (using organically and inorganically enriched media), of bacteria which utilize the phenol as a sole source of carbon and energy. The lack of chlorophenol degradation in water may be the result of extremely small degrader populations which either grow very slowly when exposed to the chemical (and thus never significantly altering the chemical concentration during the duration of an experiment) or never grow because of a nutrient limitation. Greater numbers of degraders in sediment could result in metabolism of a xenobiotic chemical without the need for growth.

Similar studies with p-nitrophenol (41), a chemical that normally requires sediment in seawater before significant degradation occurs, have shown that if the incubation time of the chemical in seawater without sediments is long enough, biodegradation will occur eventually (Fig. 5a and 5b). Again the degraders are present but unable to affect a change in PNP concentration until 600 hours incubation. The increase in degraders accompaning this degradation was similar to the increase observed originally in the sediment-containing systems. Isolation of pure cultures capable of utilizing p-nitrophenol as a sole source of carbon and energy, showed that the same bacterium was the dominant member of the populations after degradation had commenced in systems with and without sediment. Again, this evidence tends to support the idea that the microbial communities in sediment and water are essentially similar but there is more biomass (and, therefore, degraders) on sediments.

The surface-catalysis effect means that the sediment-associated bacteria that are capable of degrading a xenobiotic chemical are in some

way more active per cell than their counterparts suspended in the water. The possible concentration effect of sediment surfaces, whether for xenobiotic chemicals or for other organic or inorganic nutrients (which might promote bacterial growth or increase bacterial activity) is often suggested as an explanation for greater per cell activities. Relatively few attempts have been undertaken to experimentally verify this possibility. Novitsky (24), while demonstrating the presence of intense microbial activity at the sediment-water interface, hypothesized that the increased activity was due to larger numbers of cells rather than to a larger percentage of active cells or a difference in per cell activity. This was determined by combining heterotrophic activity measurements (tritiated glucose, glutamic acid and thymidine were used as substrates) with epifluorescence and autoradiographic direct counting of total cell numbers. His results generally agree with those of Chocair and Albright (6). However, Novitsky points out that some of his data are inconsistent with his own hypothesis; per cell activity with glutamate as a substrate was higher in sediments than in water. The opposite result was observed with glucose heterotrophic activity. Goulder (12) found maximum velocities (Vmax) of glucose mineralization in water samples taken from an estuary with a normally high load of suspended particulates, to be much higher for bacterial communities attached to suspended particulates than for free bacteria communities. Attached bacterial communities were not more active than free bacteria in estuaries with typically small suspended particulate concentrations. Her results also indicated that in general, Vmax values per cell were approximately the same for attached and free bacteria. However, during the winter samplings, per cell activities were higher in the attached communities.

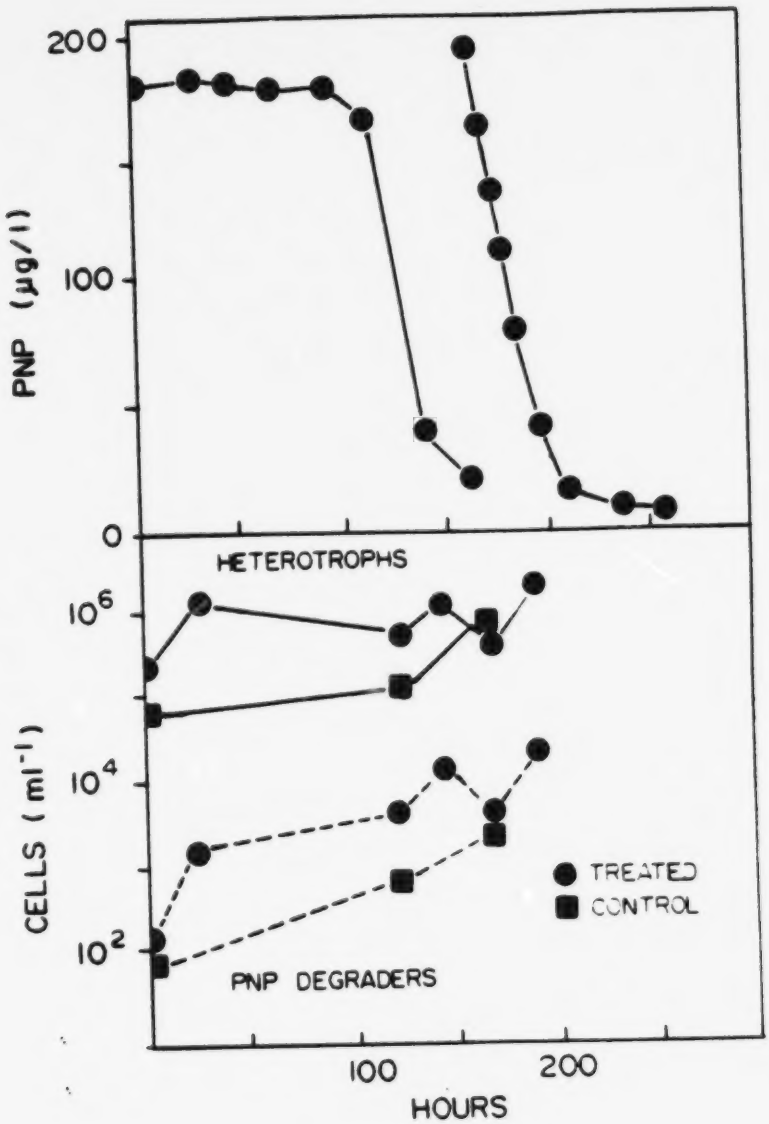


Figure 5a. Degradation of paranitrophenol (PNP) and changes in total heterotrophs and PNP degraders in flasks containing water from a freshwater pond. The flasks received an additional treatment of PNP after 600h. All data points are means of samples from triplicate flasks. Control flasks received no PNP. From Spain et al. (41).

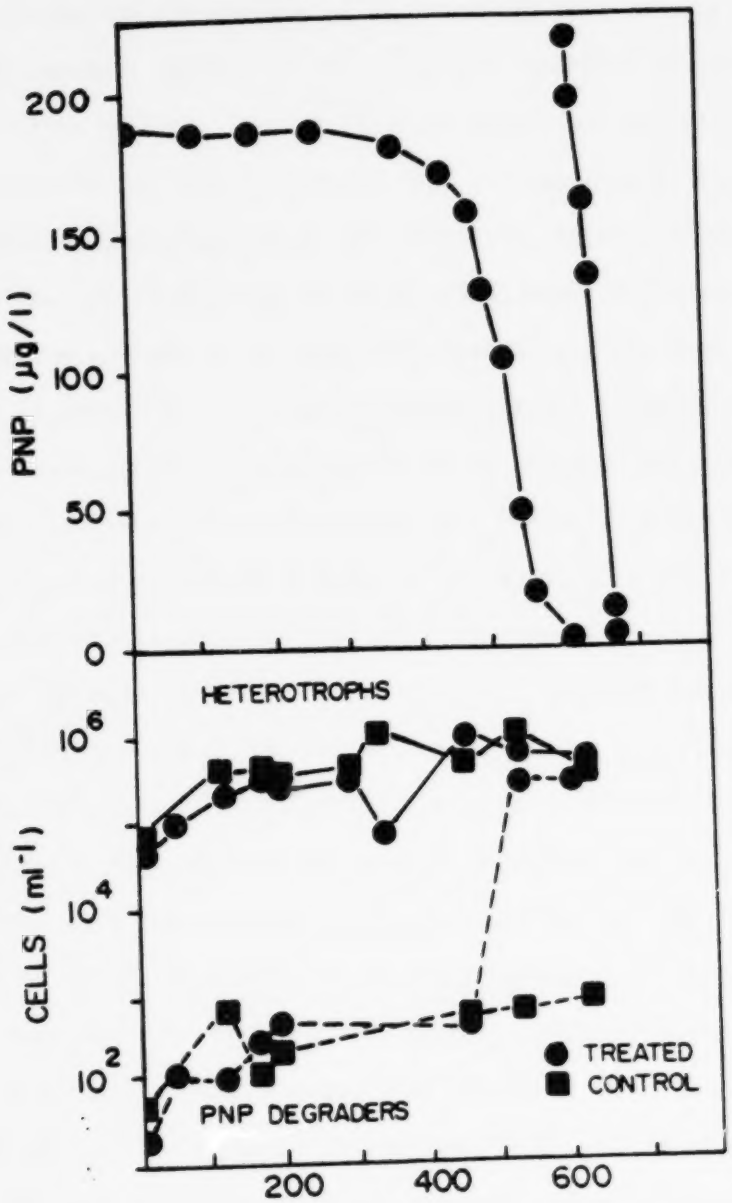


Figure 5b. Degradation of paranitrophenol and change in total heterotrophs and PNP degraders in sediment slurries (500 mg/l) from a freshwater pond. The flasks received an additional treatment of paranitrophenol after 170h. All data points are means of samples from triplicate flasks. Controls received no PNP. From Spain et al., (41).

Higher per cell activities of bacteria on sediment, if they do in fact exist, could be the consequence of an increased availability through sorption on the sediment surface of the xenobiotic chemical to the degrader population. If this is true, it must first be shown that the sorbed chemical is available for degradation and that the bacteria on the sediment are actually mediating degradation of the chemical. Several studies have examined the effect of sorptive surfaces on biodegradation processes.

Biodegradation studies in my laboratory also have shown that p-chlorophenol is persistent in seawater unless natural sediments are present. As a consequence of this observation we designed experiments to determine the tendency of sediment-associated bacteria to sluff off sediment particles and degrade chlorophenol in the water. Seawater was incubated for 150 hrs. in shake flasks under three conditions: A) by itself, B) with 500 mg/l sediment, C) with 500 mg/l sediment and 200 mg/l p-chlorophenol. At the end of the incubation period (no p-chlorophenol remained in flask C), the contents of all flasks were filtered through 3.0 µm filters (to remove sediment but not all bacteria) and the filtered water was treated with p-chlorophenol. No degradation occurred in any of the treatments (Fig. 6). The addition of fresh sediment, of course, resulted in immmediate degradation of the phenol. p-Chlorophenol degraders on the sediment either are not released into the water or if they are, they could not degrade the chemical in the seawater. Each flask was then treated with 1000 mg/l sterile autoclaved sediment. Degradation was subsequently observed in all flasks. Thus, p-chlorophenol degrading bacteria appeared to be in the water but they are unable to degrade the phenol without the presence of sediment. The results are very interesting because even bacterial communities normally associated with water (flask a)

will degrade the phenol if sterile sediment is present. Preexposure of the water to active sediment (flask b) or active sediment and p-chlorophenol (flask c) apparently increased the number of water-born p-chlorophenol degraders but degraders which require sediments for degradation. These results support the idea of xenobiotic chemical degradation by bacteria on sediment surfaces and they also demonstrate a possible interaction between the xenobiotic chemical, the bacteria, and the sediment surface to enhance biodegradation. Preliminary studies indicate the interaction does not eliminate, by sorption of the chemical to the sedimennt surface, a toxic effect of the chlorophenol. Further studies are required before a mechanism of the sediment effect can be elucidated.

I am aware of only two other studies which address the concept of "surface mediated" biodegradation by aquatic organisms. Gordon et al. (11), in a study with a periphytic marine bacterium, were able to regulate the attachment of the bacterium to hydroxyapatite particles by adjusting the number of bacteria per ml. Because hydroxyapatite was found to adsorb glutamic acid but not glucose from the experimental medium, they were able to select experimental conditions that assessed the interaction between bacterial attachment and nutrient adsorption. Their results showed that the bacterial respiration activity as measured by microcalorimetry and radiorespirometry, was not enhanced by the particles, regardless of whether the bacteria or the nutrients were sorbed to the sediment. In fact, a small decrease in respiratory activity was detected when the bacteria became attached. Their experimental design does not differeniate between a decrease due to a lower starting concentration and a decrease cause by slow desorption rates. The use of the substrate-cell-hydroxyapatite interaction (which is possibly ionic) as a model for the

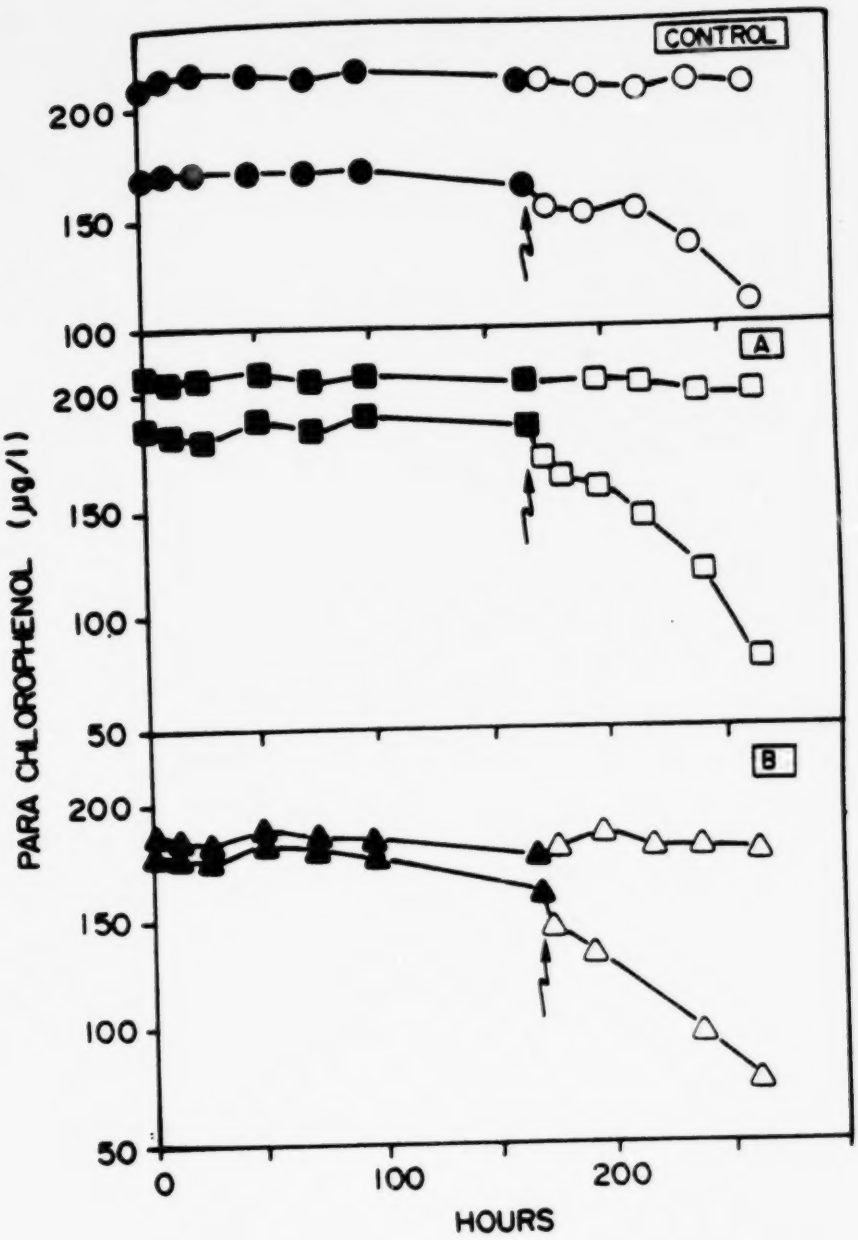


Figure 6. Concentration of p-chlorophenol in replicate flasks containing seawater previously exposed to a.) nonsterile sediments (500 mg/ℓ) and b.) nonsterile sediment (500 mg/ℓ) plus p-chlorophenol (200 µg/ℓ). No residual sediment or p-chlorophenol were present at zero hour. Control water was not preexposed. At approximately 170 hours (arrow), sterile sediments (500 mg/l) were added to one flask of each replicate and incubation continued.

effects of sorption of the biodegradation of other hydrophobic chemicals (which sorb nonionically) is also uncertain. Further studies with other bacteria are needed before the idea of a surface-catalyzed effect is rejected. The very rapid degradation of glucose and glutamic acid may be the key factor for developing conditions in which biodegradation rates approach desorption rates.

A study of Li and Digiano (25), on the other hand, resulted in exactly the opposite conclusion and indirectly supports our p-chlorophenol work. They followed the growth and biodegradation kinetics of a thin biofilm developed on three types of particles; sand, coal and granular activated carbon. The particles were incubated with secondary effluent from a sewage treatment plant and after attached-cells were observed microscopically, the particles were washed to leave only tightly attached cells. The particles, with their attached biofilm, were then placed in a special reactor which allowed the utilization of substrate by the biofilm to be measured. A typical result for phenol utilization is shown in figure 7. The exponential increase in phenol utilization rate suggests that unlimited growth of the biofilm was occurring at the end of the experiment; limitation by oxygen, substrate or hydrodynamic shear would give a constant utilization rate. Growth rate of the biofilm, as estimated from the substrate utilization rate, was shown by the authors to be independent of particle surface characteristic. Table 6 shows that growth rates with four different substrates were approximately equal for sand and coal biofilms but were considerably elevated for the activated carbon biofilm. This elevated rate was also related to particle size of the activated carbon, suggesting that the rate of resupply of the substrate to the biofilm by internal diffusion was an important rate limiting

factor. Thus the interaction between the microorganisms, the substrate and the particle surface can, in some situations, stimulate microbial activities due to biodegradation of sorbed chemical.

Although, the puzzle of understanding surface-catalyzed effects on the biodegradation of xenobiotic chemicals in sediments is being slowly resolved, the importance of further work in this area cannot be over estimated.

Assessing Biodegradation in Sediments

Biodegradation potentials, pathways, and kinetics are generally determined in shake flasks using water-sediment suspensions. Sediments in nature are of course either in a suspended state or packed in a relatively static bed. In the latter state, a very definite ordering and structuring of physical and chemical properties occurs as a function of sediment depth. A set of parameters for one layer of sediment may differ dramatically from an adjacent layer. Information obtained from laboratory flask studies must somehow be applied to these more complex natural situations. To date, little research has addressed this application or extrapolation problem. Exposure concentration estimations, particularly where a xenobiotic chemical is more rapidly biodegraded in sediments, must encompass methods which will allow extrapolation from simple test information to complex environmental situations. I believe that microcosm studies offer a very useful assessment method for this type of extrapolation. Many research investigations have convincingly shown that microcosms can reasonably simulate parts of nature (32) and as such, may be useful in obtaining information on how fate processes operate in complex situations similar to those seen in the field. For example, we have compared the fate of fenthion (Baytex) an organophosphate insecticide, in laboratory shake

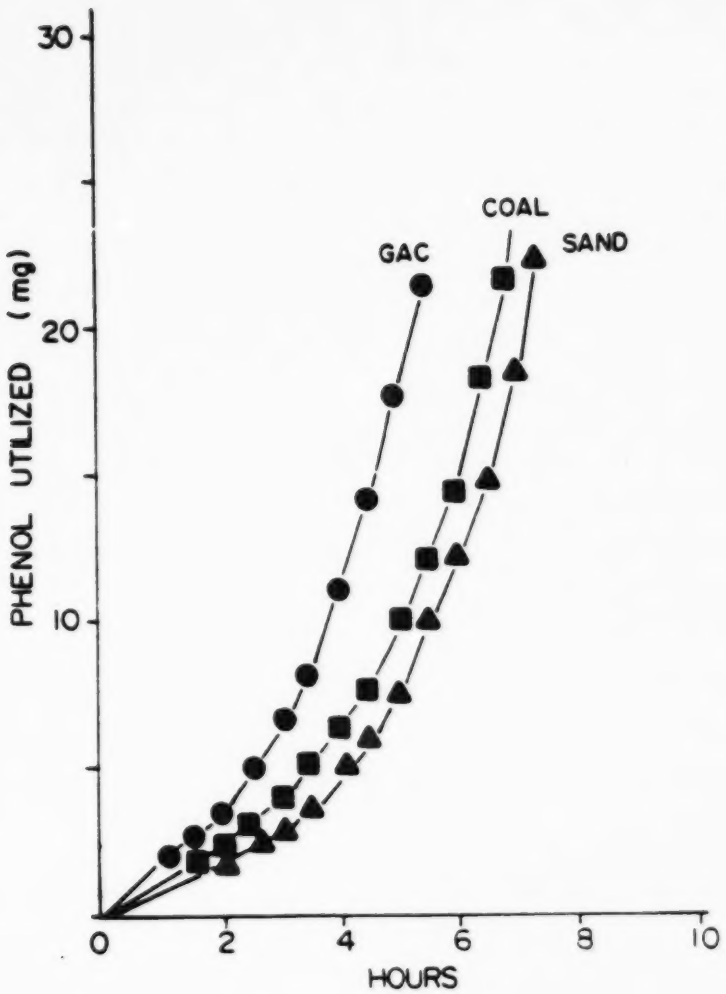


Figure 7. The progression of phenol utilization with time in an infinite batch-recycle reactor with different solid media coated with a thin biofilm. GAC is granulated activated carbon.

From Li and Digiano (25)

Table 6. Calculated specific growth rates (hr^{-1}) obtained from substrate utilization rates on different solid media.

	SOLID MEDIA[a]				
			Granular Activated Charcoal		
Substrate	Sand	Coal	26/25	35/45	50/60
O-Cresol	0.134	0.135	0.250	0.290	0.295
Acetophenone	0.103	0.083	0.178	0.208	0.225
phenol	0.253	0.278	0.323	0.345	0.396
Benzoic acid	0.188	0.204	0.247	0.280	0.340

A External surface area (cm^2) for particles is sand = 2000, coal = 2000, GAC 20/25 = 614, GAC 35/40 = 1029 and GAC 50/60 = 2000.

From Li and Digiano (25)

flasks and microcosms containing estuarine sediments. Laboratory shake flask tests in our laboratory have shown that the presence of sediments greatly stimulates (half-life = 4 days) the biodegradation of fenthion in seawater (half-life = 22 days).

To assess the importance of this sediment effect in natural systems we simulated the sediment bed of a local saltmarsh in a microcosm. This was accomplished by taking intact sediment cores from the field and placing them directly in microcosm vessels. This method preserved the sediment-water interface and the physical integrity of the sediment bed. The vessels were kept at constant temperature in the laboratory and exposed to light regimes similar to that in the field. The water column was mixed with a glass rod attached to a constant rpm motor but so as not to resuspend sediment. A set of intact cores were sterilized with formalin. Fenthion at 200 µg/l was then spiked into the water column of each vessel and its disappearance rate monitored. After a specific incubation period, overlying water was removed and the sediment cores were frozen and fractionated into 0.5 cm layers. The concentrations of dissolved and sorbed fenthion in each layer were determined. A typical example of the concentration and distribution of fenthion in sterile and nonsterile microcosms is shown in figure 8. Fenthion was lost from the water column more rapidly in active systems. Relative to the sterile microcosms, the pesticide was detected at much lower concenrations in the upper layers of sediment in the active microcosms but was found to a much greater sediment depth in active microcosms.

The factors which affect the chemical's fate in these microcosms must be carefully considered. Kinetic information on each factor must be known if the effects due to biodegradation are to be sorted out. In

the sterile systems, losses of fenthion from the water column could be the result of photolysis, hydrolysis, diffusion into sediment and sorption to sediment. Rates of hydrolysis (in water and sediment) and photolysis were therefore separately determined in standard shake flasks tests. Adsorption/desorption rates of fenthion to sediment were assumed to be instantaneous and an equilibrium partition coefficient was obtained in a batch sorption test. The partition coefficient was also assumed to be directly proportional to organic matter content in the sediment. Consequently, since the variation in organic content with depth in the sediment was known, the degree of partitioning in each sediment layer could be estimated. The amount of fenthion which rapidly adsorbs to particulate material at the sediment-water interface (i.e., independent of diffusion into sediment) was calculated from the total decrease of fenthion in the water column which occurred during the first three hours of incubation (see Figure 8a). Lastly relative diffusion rates for each sediment layer were estimated from the distribution of tritated water in the sediment of the microcosms following (96 hrs) a single application of tritiated water to the water column. This procedure allowed the pore water mixing from turbulance created by stirring the water column of the top sediment layers to be estimated. A preliminary summary of this data is given in table 7.

All of this information was then used with a simple mass balance mathematical model to calculate the fate and distribution of fenthion in the sterile microcosm. Agreement between observed and calculated values was quite good if diffusion rates in the lower sediment layers were reduced to accomodate the difference in molecular size between fenthion and tritium and if rates in the top sediment layer were increased four-fold.

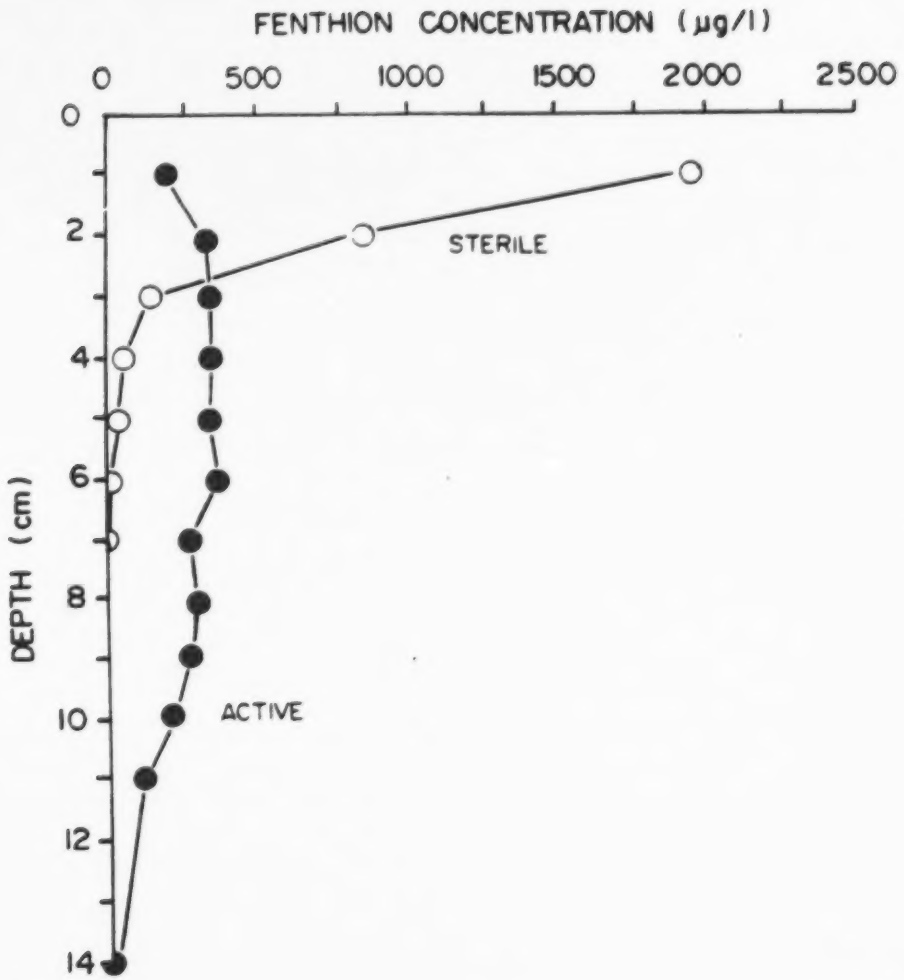


Figure 8. Fate of fenthion in sediment-water microcosms. a.) Disappearance of fenthion in the water column of sterile and nonsterile microcosms. b.) Distribution of fenthion in sterile and nonsterile sediment after 190 hours of incubation.

We currently cannot explain the need for this elevated diffusion rate to accurately fit the observed data. This diffusion information was applied to the nonsterile microcosms.

In nonsterile microcosms, two additional processes affect the fate of the pesticde; biodegradation and bioturbation. Bioturbation was recognized because of considerably higher concentrations of tritium found in the sediment (following addition of tritiated water to the water column) as compared to the sterile microcosms. Diffusion rates in the model were adjusted in an attempt to accomodate the additional pore water mixing probably caused by bioturbation. This adjustment, however, was not sufficient to account for the high concentration of fenthion observed in the lower sediment layers of the nonsterile microcosm. Consequently, by assuming that bioturbation redistributed sediment particles containing sorbed fenthion, the model was run with several different diffusion rates in the sediment layers above 8 cm (bioturbation zone) until model calculations agreed approximately with the observed fenthion concentrations. Calculated and observed concentrations, under conditions in which biodegradation in the sediments was not considered, are shown in figure 9 (curve B).

The effects of biodegradation were then evaluated. Second-order biodegradation rate constants (i.e., with respect to substrate concentration and degrader biomass concentration) for water and sediment were determined from shake flask studies. Degradation in sediment was shown to be directly related to the concentration of organic detritus. Detritus was used as a surrogate measure of degrader biomass concentration in sediment; sand was assumed to be devoid of surface microorganisms which could degrade fenthion. Since the sand/detritus ratio varied with each sediment layer, biodegradation in the model was adjusted accordingly. Biodegradation of dissolved and

Table 7. Preliminary kinetic information used to describe the fate and distribution of fenthion in sediment-water microcosms.

Parameter[a]	Microcosms	
	Sterile	Nonsterile
Diffusion Rates ($cm^2/sec \times 10^{-5}$)	40[b] 2[c] 1[d]	140[b] 40[c] 8[d] 1.4[e]
Partition coefficicent ($\mu g/kg\ (\mu g/\ell)^{-1}$)	700	700
Initial fenthion conc. in sediment ($\mu g/\ell$)	707	1023
Photolysis rate constant (hr^{-1})	0.0007	0.0013
Abiotic degradation rate constant, water (hr^{-1})	0.0001	0.0001
Abiotic degradation rate constant, sediment (ℓ/gm hr)	0.00006	0.00006
Biodegradation rate constant, water (hr^{-1})[f]	0	0.0013
Biodegradation rate constants, sediment (ℓ/gm hr)	0	0.00004

[a] Parameters derived from shake flask tests using water and water-sediment slurries

[b] Between water and the first sediment layer (0.5 cm)

[c] Between first sediment layer and the second sediment layer

[d] Between seventh sediment layer and the eighth sediment layer

[e] Between all remaining sediment layers

[f] First order rate constant used because biomass was equal in flask and microcosm water

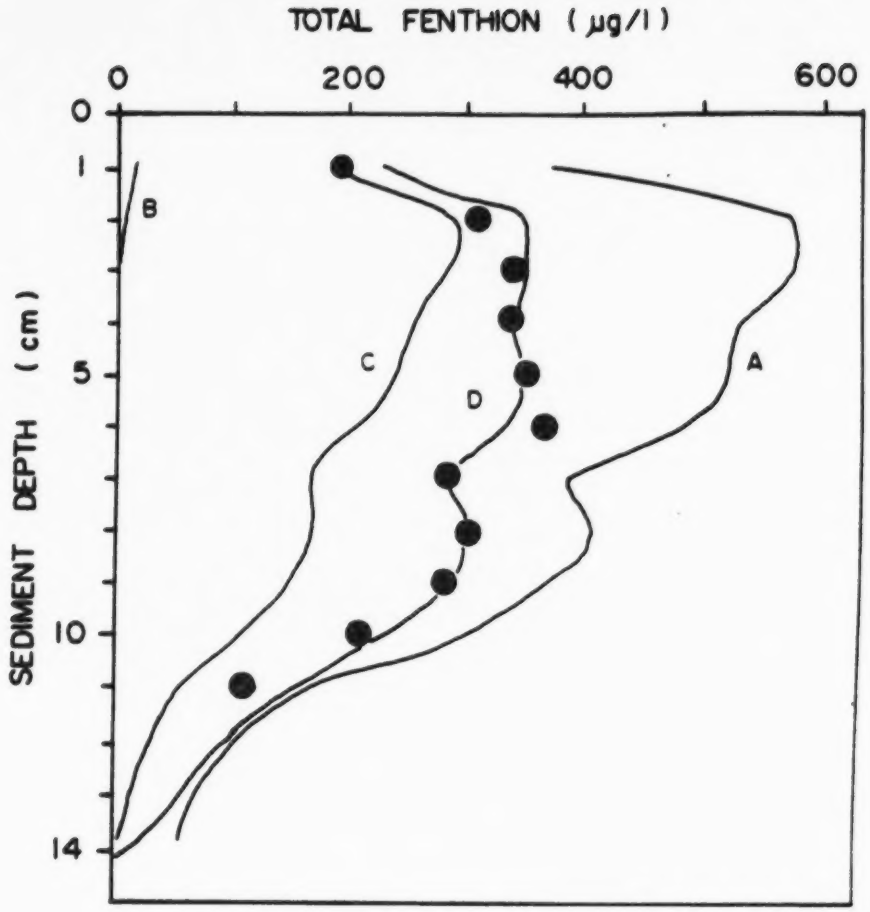


Figure 9. Observed data points and calculated (solid lines) concentration of fenthion in the sediment of salt marsh microcosms. A.) Curve based on model fit to fenthion distribution in sediment below 8 cm. Bioturbation zone is assumed to be approximately 8 cm deep. Biodegradation rate at all depths was set to zero, B.) Biodegradation rate in all layers set to equal rate from shake flask studies, C.) Biodegradatin rate in all layers set to 1/40 of rate from flask studies, D.) Biodegradation rate graduated from the top sediment layer (fastest rate) to 8 cm depth and rate set at zero in all layers below 8 cm depth.

sorbed chemical was considered in the model. The calculated distribution of fenthion in sediment using the sediment biodegradation rate constant derived from the shake flask studies is shown in figure 9 (curve B). Obviously, the degradation rate is too high relative to what is actually occurring in the sediment bed. Arbitrarily reducing the rate constant 40 fold improves the model fit to the data (curve C). The best fit, however, is obtained when the degradation rate is adjusted to zero below the 8 cm depth in the sediment and graduated above 8 cm so that the rate is fastest in top layer and then decreases with depth (curve D). Specific depth-related environmental conditions in the intact sediment bed, such as redox potential, dissolved oxygen concentration and/or inorganic nutrient concentrations, could be responsible for the apparent range of degradaton capabilities.

Asssessing the biodegradation rates of xenobiotic chemicals in the natural sediments is undoubtedly a complicated process. Although the microcosms appear to be an excellent method for carrying out these assessments, our work indicates the importance of a thorough knowledge of all relevant fate processes in these systems. Using a mathematicl model to integrate these fate processes is an excellent mechanism for revealing where information is insufficient and for suggesting where new experiments are needed. Several other modeling studies, using soil systems (46), ponds (26, 35) and streams (10), have likewise shown the importance of the holistic approach and the extent of our inability to assess biodegradation process in intact sediment/soil systems. Bioturbation, also greatly affects chemical distribution in sediment and consequently, biodegradation. This aspect has been only minimally characterized. Studying sediments in shake flasks also may give exaggerated estimates of

biodegradation rates and the flask tests may not indicate the possible relative differences in rates of degradation with depth. Additional work to quantitatively relate biodegradation kinetics in intact and suspended sediment tests is needed since our knowledge therein currently limits, significantly, our ability to assess biodegradation in natural aquatic systems.

RECOMMENDATIONS

1. Extensive efforts should be undertaken to more fully characterize the biodegradation capabilities of microbial communities associated with sediments. Rates and pathways of biodegradation should be established wherever possible.
2. Little work has been undertaken to characterize the enhancement or inhibition of degradation as the result of a chemicals interaction with the sediment surface and its associated microflora. This work needs to be expanded for a series of hydrophobic chemicals such as chlorinated biphenyls or PAH's.
3. More research should be applied to specifically characterizing the rates of xenobiotic chemical biodegradation in sediment beds contained in field studies or microcosms, particularly where it entails using laboratory data for predictive purposes.
4. Kinetic expression(s) for the biodegradation of chemicals in sediments need to be further developed and validated.
5. The biodegradation of xenobiotic chemicals needs to be examined more carefully in the context of natural organic matter transformations which occur in sediments. Special attention should be made of the factors which control these transformation processes both in time and in space. Factors such as bioturbation and oxidation-reduction gradients are particularly important for pollutant degradation.

ACKNOWLEDGMENTS

I thank Dr. Paul Lefcourt for permission to include his unpublished data in this paper. I also thank Ms. Ellen O'Neill and Ms. Carol Monti for their technical assistance with the fenthion and chlorophenol experiments, respectively. The helpful discussions with Rick Cripe, Len Mueller, and especially John Connolly, who provided assistance with the mathematical expressions, are greatly appreciated. Don Ahearn, Paul Lefcourt, Rob Shrimp, John Rodgers and Chuck Steen are thanked for their review of this paper. Part of the research information presented in this paper was provided as part of cooperative agreement CR809370 between the Environmental Protection Agency, Gulf Breeze and Georgia State University.

LITERATURE CITED

1. Baker, M.D., C.I., Mayfield and W.E. Inniss. 1980. Degradation of chlorophenols in soil, sediment, and water at low temperature. Water Res. 14:1765-1771.

2. Bourquin, A.W., M.A. Hood and R.L. Garnas. 1977. An Artificial Microbial Ecosystem for Determining Effects and Fate of Toxicants in a Salt-Marsh Environment. Dev. Ind. Microbiol. 18:185-191.

3. Bull, A.T. 1980. Biodegradation:Some attitudes and strategies of microorganisms and microbiologists. In: Contemporary Microbial Ecology, D.C. Ellwood, et al. (eds.). Academic Press Inc., London. pp. 107-136.

4. Caplan, J.A., A.R. Isensee and J.O. Nelson. 1984. Fate and effects of [^{14}C] fenvalerate in a tidal saltmarsh sediment ecosystem model. J. Agr. Food Chem. 32:166-171.

5. Chapman, R.A., C.M. Tu, C.R. Harris and C.Cole. 1981. Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil. Bull. Environ. Contam. Toxicol. 26:513-519.

6. Chocair, J.A. and L.J. Albright. 1981. Heterotrophic activities of bacterioplankton and bacteriobenthos. Can. J. Microbiol. 27:259-266.

7. Clark, J.M. and F. Matsumura. 1979. Metabolism of toxaphene by aquatic sediments and a camphor-degrading pseudomonad. Arch. Environ. Contam. Toxicol. 8:285-298.

8. DeLaune, R.D., R.P. Gambrell and K.S. Reddy. 1983. Fate of pentachlorophenol in estuarine sediment. Environ. Pollut. 6:297.

9. Fortmann, L. and A. Rosenberg. 1984. Fate of Caprolactam in the aquatic environment. Chemosphere 13:53-65.

10. Games, L.M. 1982. Field validation of exposure analysis modeling system (EXAMS) in a flowing stream. In "Modeling the Fate of Chemicals in the Aquatic Environment," K.L. Dickson, A.W. Maki and J. Cairns Jr. (eds.). Ann Arbor Science, Ann Arbor Michigan. pp. 325-346.

11. Gordon, A.S., S.M. Gerchakov, and F.J. Millero. 1983. Effects of inorganic particles on metabolism by a perphytic marine bacterium. Appl. Environ. Microbiol. 45:411-417.

12. Goulder, R. 1977. Attached and free bacteria in an estuary with abundant suspended solids. J. Appl. Bacteriol. 43:399-405.

13. Graetz, D.A., G. Chesters, T.C. Daniel, L.W. Newland, and G.B. Lee. 1970. Parathion degradation in lake sediments. J. Water. Pollut. Control Fed. 2:R76-R94.

14. Griffiths, R.P., S.S. Hayasaka, T.M. McNamara, and R.Y. Morita. 1978.

Relative microbial activity and bacterial concentrations in water and sediment samples taken in the Beaufort Sea. Con. J. Microbiol. 24:1217-1226.

15. Herbes, S.E. 1981. Rate of microbial transformation of polycyclic aromatic hydrocarbons in water and sediments in the vicinity of a coal-coking waste water discharge. Appl. Environ. Microbiol. 41:20-28.

16. Holm, T.R., M.A. Anderson, R.R. Stanforth and D.G. Iverson. 1980. The influence of adsorption on the rates of microbial degradation of arsenic species in sediments. Limnol. Oceanogr. 25:23-30.

17. Johnson, B.T. and W. Lulves. 1975. Biodegradation of di-n-butylphthalate and di-2-ethylhexylphthalate in freshwater hydrosoil. J. Fish. Res. Bd. Can. 32:333-339.

18. Jones, T.W., W.M. Kemp. J.C. Stevenson, and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632-638.

19. Karl, D.M. 1982. Microbial transformations of organic matter at oceanic interfaces: a review and prospectus. EOS Revista Espanola de Entomolgia. 63:1073-1079.

20. Kong, H. and G.S. Sayler. 1983. Degradation and total mineralization of monohalogenated biphenyls in natural sediment and mixed bacterial culture. Appl. Environ. Microbiol. 46:666.

21. Larson, R.J. 1984. Kinetic and Ecological Approaches for Predicting Biodegradation Rates of Xenobiotic Organic Chemicals in Natural Ecosystems. In "Current Perspectives in Microbial Ecology." M.J. Klug and C.A. Reddy (eds.) American Society for Microbiology, Washington, DC. pp. 677-686.

22. Larson, R.J. and R.D. Vashon. 1983. Adsorption and biodegradation of cationic surfactants in laboratory and environmental systems. Dev. Ind. Microbiol. 24:425-434.

23. Lee, R.F. and C. Ryan. 1979. Microbial degradation of organochlorine compounds in estuarine water and sediments. In: Microbial Degradation of Pollutants in Marine Environments, A.W. Bourquin and P.H.Pritchard (eds.). U.S. Environmental Protection Agency. EPA-600/9-79-012. pp. 443-450.

24. Lee, G.F., R.A. Hughes, and G.D. Veith. 1977. Evidence for partial degradation of toxaphene in the aquatic environment. Water, Air, Soil Pollut. 8:479-484.

25. Li, A.Y. and F.A. DiGiano. 1983. Availability of sorbed substrate on microbial degradation on granular activated carbon. J. Water Pollut. Control Fed. 55:392-399.

26. Marshall, W.K. and J.R. Roberts. 1971. Simulation modelling of the distribution of pesticides in ponds. Scientific Criteria for Environmental Quality. 2:253-278. Nat. Res. Council, NRCC/CNRR 16073, Canada.

27. Miyazaki, S., H. C. Sikka and R.S. Lynch. 1975. Metabolism of dichlobenil by microorganisms in the aquatic environment. J. Agric. Food. Chem. 23:365-368.

28. Novitsky, J.A. 1983a. Heterotrophic activity throughout a vertical profile of seawater and sediment in Halifax Harbor, Canada. Appl. Environ. Microbiol. 45:1753-1760.

29. Novitsky, J.A. 1983b. Microbial activity at the sediment-water interface in Halifax Harbor, Canada. Appl. Environ. Microbiol. 45:1761-1766.

30. Perez, K.T., E.W. Davey, N.F. Lackie, G.E. Morrison, P.G. Murphy, A.E. Soper and D.L. Winslow. 1985. Environmental assessment of a phthalate ester, di(z-ethyl hexyl)phthalate (DEHP), derived from a marine microcosm. In "Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802" W.E. Bishop, R.D. Cardwell and B.B. Heidolph, eds. Am. Soc. Testing and Materials, Philadelphis (in press).

31. Pignatello, J.J., M.M. Martinson, J.G. Steiert, R.E. Carlson, and R.L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. Appl. Environ. Microbiol. 46:1024-1031.

32. Pritchard, P.H. and A.W. Bourquin. 1983. The use of microcosms for evaluation of interactions between pollutants and microorganisms. In: Advances in Microbial Ecology, Volume 7. C.C. Marshall (ed.), Plenum Press, New York, NY. pp. 133-215.

33. Pritchard, P.H., A.W. Bourquin, H.L. Fredrickson, and T. Maziarz. 1979. System design factors affecting environmental fate studies in microcosms. Workshop: Microbial Degradation of Pollutants in Marine Environments, A.W. Bourquin and P.H. Pritchard (eds.). U.S. Environmental Protection AGency, Environmental Research Laboratory, Gulf Breeze, FL. pp. 251-272.

34. Rawn, G.P., G.R. Webster and D.C. Muir. 1982. Fate of permethrin in model outdoor ponds. J. Environ. Sci. Hlth. B17:463-486.

35. Rodgers, J.H., K.L. Dickson, F.Y. Saleh and C.A. Staples. 1983. Use of microcosms to study transport, transformation and fate of organics in aquatic systems. Environ. Toxicol. Chem. 2:155-167.

36. Schaurte, W., J.P. Lay, W. Klein and F. Korte 1982. Long-term fate of organochlorine xenobiotics in aquatic ecosystems. Ecotoxicol. Environ. Safety 6:560-569.

37. Sikka, H.C. and C.P. Rice. 1973. Persistence of endothall in aquatic environment as determined by gas-liquid chromatography. J. Agr. Food. Chem. 21:842-846.

38. Simsiman, G.V. and G. Chesters. 1975. Persistence of endothall in the aquatic environment. Water, Air, Soil Pollut. 4:399-413.

39. Simsiman, G.V. and G. Chesters. 1976. Persistence of diquat in the aquatic environment. Water Res. 10:105-112.

40. Smith, S. and G.H. Willis. 1978. Disappearance of residual toxaphene in a Mississippi delta soil. Soil Sci. 126:87-93.

41. Spain, J.C., P. Van Veld, C.A. Monti, P.H. Pritchard, C.R. Cripe. 1984. Comparison of p-nitrophenol degradation in field and laboratory test systems. Appl. Environ. Microbiol. 48:944-950.

42. Steen, W.C., D.F. Paris, and G.L. Baughman. 1980. Effects of sediment sorption on microbial degradation of toxic substances. In: Contaminants and Sediments, R.A. Barker (ed.). Ann Arbor Science, Ann ARbor. MI. pp. 477-482.

43. Subba-Rao, R.V. and M. Alexander. 1982. Effect of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. 44:659-668.

44. Van Veld, P.A. and J.C. Spain. 1982. Degradation of selected xenobiotic compounds in three types of aquatic test systems. Chemosphere 12:86-97.

45. Walker, W.W., C.R. Cripe, P.H. Pritchard, and A.W. Bourquin. 1984. Biotic and abiotic degradation of dibutylphthalate in estuarine and freshwater environments. In press, Chemosphere.

46. Walker, A. 1978. Simulation of the persistence of eight soil applied herbicides. Weed Res. 18:305-310.

47. Ward, C.T. and F. Matsumura. 1978. Fate of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch. Environ. Contam. Toxicol. 7:349-352.

48. Weber, J.B. and H.D. Coble. 1968. Microbial decomposition of diquat adsorbed on montmorillonite and kaolinite clays. J. Agr. Food Chem. 16:475-478.

49. Williams, R. and T. Bidleman. 1978. Toxaphene degradation in estuarine sediments. J. Agr. Food Chem. 26:280-283.